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L-Tryptophan Reacts with Naturally Occurring and Food-Occurring Phenolic Aldehydes To Give Phenolic Tetrahydro-β-carboline Alkaloids: Activity as Antioxidants and Free Radical Scavengers

TOMAS HERRAIZ,* JUAN GALISTEO, AND CRISTINA CHAMORRO

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

The reaction between the essential amino acid L-tryptophan and flavoring or naturally occurring phenyl and phenolic aldehydes was studied, and the alkaloidal reaction products were characterized by NMR and HPLC-MS. Benzaldehyde, vanillin, syringaldehyde, salicylaldehyde, and anisaldehyde condensed with L-tryptophan in aqueous−acidic media affording the corresponding phenolic tetrahydro-β-carboline-3-carboxylic acid as two diastereoisomers, 1*S*,3*S*-*cis* and 1*R*,3*S*-*trans*. With the exception of benzaldehyde, the rest of the aldehydes needed heating conditions (70 °C) to significantly form tetrahydro-β-carbolines over time with the cyclization highly favored at low pH. This suggests a likely formation of these compounds under conditions that may occur in foods, food processing, or cooking. The new phenolic tetrahydro-β-carboline alkaloids were assayed, for the first time, for their activity as free radical scavengers and antioxidants and showed good antioxidant properties with Trolox equivalent antioxidant capacity (TEAC) values much higher than those of ascorbic acid and the water soluble vitamin E analogue, Trolox, in the 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay.

KEYWORDS: Tryptophan; alkaloids; β-carbolines; tetrahydro-β-carbolines; aldehydes; phenolic compounds; antioxidant; ABTS

INTRODUCTION

L-Tryptophan is an essential amino acid that plays an important role in nature, serving as a building block or functional unit in proteins and taking part in the biosyntheses of hormones and brain neurotransmitters such as serotonin. Tryptophan influences several physiological processes such as brain function, hypertension, and behavior (*1*). Tryptophan-derived tetrahydro-β-carboline and β-carboline alkaloids have been increasingly found in mammalian tissues and fluids, as well as in many foods of different origins (*2*). This finding is of further interest because these compounds are of physiological significance. They might function as neuromodulators via effects on monoamine oxidase (MAO), neuroamine uptake, and benzodiazepine receptor binding (*3*−*8*). Some β-carbolines might act as comutagenic substances (*9*), precursors of *N*-nitroso compounds (*10*, *11*), and toxic compounds (*12*−*14*).

Tetrahydro-β-carbolines and β-carbolines have been increasingly found in foods, alcoholic and nonalcoholic drinks, and fruit-derived products (*15*−*21*). Indeed, β-carbolines are naturally occurring substances produced during food production, processing, and storage. It is now well established that these alkaloids may occur under mild conditions in foods from a Pictet−Spengler condensation of indoleamines such as L-tryptophan and short aliphatic aldehydes (*22*). Many aldehydes may appear in foods during production and processing or are added to increase flavor (*23*). This is the case of phenyl and phenolic aldehydes such as benzaldehyde, vanillin, salicylaldehyde, syringaldehyde, and anisaldehyde that can either occur naturally in foods or be added as flavoring agents. These aromatic aldehydes might produce novel tetrahydro-β-carbolines in foods during processing or cooking through a condensation reaction with L-tryptophan. The present work was aimed to check this assumption, and as a result we show the formation of several phenolic tetrahydro-β-carbolines in aqueous systems as tryptophan−phenolic aldehyde condensation products. In addition, a potential antioxidant activity as free radical scavengers of these new alkaloids containing phenolics substituents within a tetrahydro-β-carboline moiety is also highlighted, showing that these compounds may be good antioxidants.

MATERIALS AND METHODS

L-Tryptophan was obtained from Sigma Chemical Co. (St. Louis, MO), and aldehydes were purchased from different commercial sources. ^{1}H NMR spectra were recorded with a Varian XL-400 spectrometer operating at 400 MHz with Me_4Si as internal standard and the chemical shifts calibrated by using the solvent signal. ^{13}C NMR spectra were

* Author to whom the correspondence should be addressed (fax 34-915644853; e-mail therraiz@ifi.csic.es).

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recorded using a Varian XL-400 spectrometer operating at 100 MHz with Me_4Si as internal standard. For signal assignments, NOESY, DEPT, HMQC, and HMBC experiments were carried out.

Analytical RP-HPLC was performed as previously described (*16*). Briefly, a 150 mm × 3.9 mm, 4 μm, Nova-Pak C18 column (Waters, Milford, MA) was used for separation. Chromatographic conditions were as follows: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B); 0% (100% A) to 32% B in 8 min, and then 90% B at 18 min. The flow rate was 1 mL/min, the column temperature was 40 °C, and the injection volume was 20 μL. Two systems coupled in series were used for detection: fluorescence detection (270 nm for excitation and 343 nm for emission) and absorbance at 254 nm.

RP-HPLC-MS (electrospray ionization, ESI) was accomplished on a 150 mm × 3.9 mm, 4 μm, Nova-Pak C18 column (Waters), by using an HPLC-MSD series 1100 (Hewlett-Packard) (electrospray-positive ion mode). Eluents were, A, formic acid (0.5%), and B, 0.5% formic acid in acetonitrile; 0−60% B in 40 min. Flow = 0.70 mL/min. Cone voltage = 50 V. Mass range = 50−1000 uma.

Reaction of L-Tryptophan with Aromatic Aldehydes To Give Tetrahydro-β-carbolines. As a general procedure, L-tryptophan (306 mg, 1.5 mmol) dissolved in 0.05 N H_2SO_4 (13 mL) was stirred with the corresponding aromatic or phenolic aldehyde (1.65 mmol) to give a precipitate that was filtered off and washed with cold water. The filtrate was evaporated under vacuum to dryness. The reaction was followed by RP-HPLC, and the final products were analyzed in NMR, MS, and HPLC-MS. Synthesized 1,3-disubstituted tetrahydro-β-carbolines contained two isomers with configurations of 1*S*,3*S*-*cis* and 1*R*,3*S*-*trans* (*24*, *25*). The cis/trans ratios of the mixed isomers measured by using the ^{1}H NMR signals were in good agreement with those obtained by RP-HPLC that allowed a good separation of the two diastereoisomers. The following alkaloids were obtained.

*(1S,3S)- and (1R,3S)-1-Phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**1a**-cis and **1b**-trans).* Following the general procedure, L-tryptophan (1.5 mmol) and benzaldehyde (1.6 mmol) were allowed to react at room temperature for 9 days, and a precipitate was collected to afford **1a** (245 mg, 56%) as a white solid. The filtrate was evaporated to afford **1a**:**1b** (1.2:1) as a syrup. **1a**: ^{1}H NMR (CD_3OD + TFA) δ 3.53−3.79 (m, 2H, H-4), 4.84 (dd, 1H, H-3, $J_{3,4a}$ = 12.0 Hz, $J_{3,4b}$ = 5.2 Hz), 6.11 (s, 1H, H-1), 7.23−7.84 (m, 9H, indol, Ph); ^{13}C NMR (CD_3OD + TFA) δ 23.51 (C-4), 58.90 (C-3), 59.81 (C-1), 109.20 (C-4a), 112.43 (C-8), 119.16 (C-5), 120.60 (C-6), 123.73 (C-7), 127.14 (C-4b), 128.32 (C-9a), 130.30, 131.15, 131.47, 135.64 (Ph), 138.76 (C-8a), 172.19 (COOH); RP-HPLC-ESI-MS (Rt = 17.09 min) $(M + H)^+$ 293, (M + H − 73) 220. **1b**: ^{1}H NMR (CD_3OD) δ 4.35 (dd, 1H, H-3, $J_{3,4a}$ = 7.7 Hz, $J_{3,4b}$ = 4.9 Hz), 6.28 (s, 1H, H-1); ^{13}C NMR (CD_3OD + TFA) δ 55.06 (C-3), 56.77 (C-1); other signals as **1a**; RP-HPLC-ESI-MS (Rt = 18.2 min) $(M + H)^+$ 293, (M + H − 73) 220.

*(1S,3S)- and (1R,3S)-1-(o-Hydroxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**2a**-cis and **2b**-trans).* Following the general procedure, L-tryptophan (1.5 mmol) and salicylaldehyde (1.8 mmol) (Fluka) were allowed to react first at 40° C overnight and then at 70 °C for 6 days, and a precipitate was collected of **2a**:**2b** (1:2.5) (347 mg, 75%). **2b**: ^{1}H NMR (CD_3OD + TFA) δ 3.52, 3.74 (m, 2H, H-4), 4.52 (dd, 1H, H-3, $J_{3,4a}$ = 8.6 Hz, $J_{3,4b}$ = 5.6 Hz), 6.57 (s, 1H, H-1), 7.03−7.75 (m, 8H, indol and Ph); ^{13}C NMR (CD_3OD + TFA) δ 23.43 (C-4), 52.22 (C-1), 53.51 (C-3), 107.73 (C-4a), 112.55 (C-8), 116.67 (Ph), 119.11 (C-5), 120.76 (C-6), 121.44 (Ph), 123.89 (C-7), 127.74 (C-4b), 132.84 (C-9a), 134.84 (Ph), 138.69 (C-8a), 157.31 (Ph-OH), 171.18 (COOH); RP-HPLC-ESI-MS (Rt = 18.41 min) $(M + H)^+$ 309, (M + H − 73) 236. **2a**: ^{1}H NMR (CD_3OD + TFA) δ 4.80 (dd, 1H, H-3, $J_{3,4a}$ = 11.9 Hz, $J_{3,4b}$ = 5.1 Hz), 6.46 (s, 1H, H-1); ^{13}C NMR (CD_3OD + TFA) δ 54.82 (C-1), 57.86 (C-3); other signals similar to **2b**; RP-HPLC-ESI-MS (Rt = 16.8 min) $(M + H)^+$ 309, (M + H − 73) 236.

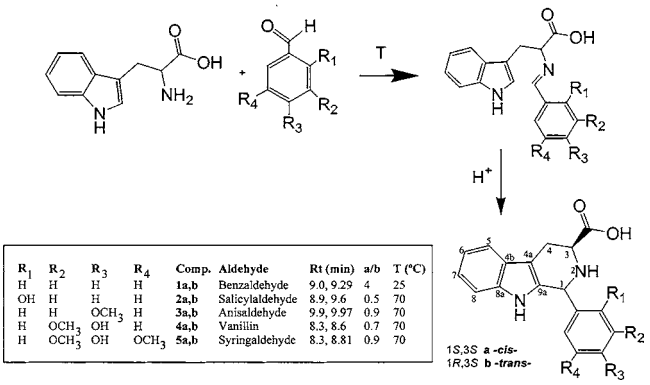
*(1S,3S)- and (1R,3S)-1-(p-Methoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**3a**-cis and **3b**-trans).* Following the general procedure, L-tryptophan (1.5 mmol) and anisaldehyde (1.65 mmol) (Carlo Erba) were allowed to react at 40 °C overnight and then at 70 °C for 6 days, and a solid precipitate was collected of **3a**:**3b** (1:2.1) (135 mg, 28%). A mixture of **3a**:**3b** (1.3:1) was obtained as a syrup after evaporation of the filtrate. **3b**: ^{1}H NMR (CD_3OD) δ 3.81 (m, 2H, H-4), 4.00 (s, 3H, OCH_3), 4.67 (dd, 1H, H-3, $J_{3,4a}$ = 7.6 Hz, $J_{3,4b}$ = 5.8 Hz), 6.24 (s, 1H, H-1), 7.12−7.81 (m, 8H, indol and Ph); ^{13}C NMR (CD_3OD) δ 23.30 (C-4), 53.03 (C-3), 55.97 (OCH_3), 56.58 (C-1), 107.45 (C-4a), 112.49 (C-8), 115.69 (Ph), 119.20 (C-5), 120.74 (C-6), 122.87 (Ph), 123.90 (C-7), 126.87 (C-4b), 128.39 (C-9a), 132.59 (Ph), 138.74 (C-8a), 162.74 (Ph), 170.98 (COOH); RP-HPLC-ESI-MS (Rt = 19.4 min) $(M + H)^+$ 323, (M + H − 73) 250. **3a**: ^{1}H NMR (CD_3OD) δ 4.80 (dd, 1H, H-3, $J_{3,4a}$ = 12.1 Hz, $J_{3,4b}$ = 5.3 Hz), 6.08 (s, 1H, H-1); ^{13}C NMR (CD_3OD) δ 57.47 (C-3), 59.57 (C-1); other signals as in **3b**; RP-HPLC-ESI-MS (Rt = 18.67 min) $(M + H)^+$ 323, (M + H − 73) 250.

*(1S,3S)- and (1R,3S)-1-(4′-Hydroxy-3′-methoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**4a**-cis and **4b**-trans).* Following the general procedure, L-tryptophan (1.5 mmol) and vanillin (1.65 mmol) (Merck) were allowed to react at 40 °C overnight and then at 70 °C for 8 days to afford **4a**:**4b** (1.1:1) (259 mg, 51%) as a solid precipitate. The filtrate was evaporated to give **4a**:**4b** (1: 2.75) as a syrup. **4b**: ^{1}H NMR (CD_3OD + TFA) δ 3.49 (m, 2H, H-4), 3.99 (s, 3H, OCH_3), 4.30 (dd, 1H, H-3, $J_{3,4a}$ = 8.1 Hz, $J_{3,4b}$ = 5.9 Hz), 6.16 (s, 1H, H-1), 6.94−7.74 (m, 7H, indol and Ph); ^{13}C NMR (CD_3OD + TFA) δ 23.69 (C-4), 53.30 (C-3), 56.50 (OCH_3), 57.14 (C-1), 107.54 (C-4a), 112.62 (C-8), 114.17, 116.77 (Ph), 119.20 (C-5), 120.84 (C-6), 124.04 (C-7), 124.50, 126.08 (Ph), 127.12 (C-4b), 129.40 (C-9a), 138.75 (C-8a, Ph), 149.88 (Ph), 171.14 (COOH); RP-HPLC-ESI-MS (Rt = 16.4 min) $(M + H)^+$ 339, (M + H − 73) 266. **4a**: ^{1}H NMR (CD_3OD + TFA) δ 4.35 (dd, 1H, H-3, $J_{3,4a}$ = 12.0 Hz, $J_{3,4b}$ = 5.1 Hz), 5.91 (s, 1H, H-1); ^{13}C NMR (CD_3OD + TFA) δ 57.66 (C-3), 60.29 (C-1); other signals as **4b**; RP-HPLC-ESI-MS (Rt = 16.0 min) $(M + H)^+$ 339, (M + H − 73) 266.

*(1S,3S)- and (1R,3S)-1-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**5a**-cis and **5b**-trans).* Following the general procedure, L-tryptophan (1.5 mmol) and syringaldehyde (1.65 mmol) (Aldrich) were allowed to react at 70 °C for 6 days, and a precipitate was collected of **5a**:**5b** (1.1:1) (254 mg, 46%). A mixture (1:1.2) of **5a**:**5b** was obtained following evaporation of the filtrate. **5a**: ^{1}H NMR (CD_3OD + TFA) δ 3.77 (m, 2H, H-4), 3.97−4.02 [2s, 6H, $(OCH_3)_2$], 4.80 (dd, 1H, H-3, $J_{3,4a}$ = 12.1 Hz, $J_{3,4b}$ = 5.3 Hz), 6.01 (s, 1H, H-1), 6.85, 6.98 (2s, 2H, Ph), 7.2−7.65 (m, 4H, indol); ^{13}C NMR (CD_3OD + TFA) δ 23.73 (C-4), 56.91 (OCH_3), 57.69 (C-3), 60.58 (C-1), 108.30 (C-4a), 108.47 (Ph), 112.56 (C-8), 119.25 (C-5), 120.84 (C-6), 124.04 (C-7), 124.45 (Ph), 127.16 (C-4b), 129.43 (C-9a), 138.78 (C-8a), 149.73, 158.84, 160.24 (Ph), 171.06 (COOH); RP-HPLC-ESI-MS (Rt = 15.95 min) $(M + H)^+$ 369, (M + H − 73) 296. **5b**: ^{1}H NMR (CD_3OD + TFA) δ 4.72 (m, 1H, H-3), 6.22 (s, 1H, H-1); ^{13}C NMR (CD_3OD + TFA) δ 53.60 (C-3), 57.37 (C-1); other signals as **5a**; RP-HPLC-ESI-MS (Rt = 16.8 min) $(M + H)^+$ 369, (M + H − 73) 296.

Formation of Phenolic Tetrahydro-β-carbolines as a Function of pH and Temperature. Solutions of L-tryptophan (300 mg/L) at different pH values [pH 1 (HCl, NaCl) and 50 mM buffer phosphate solutions of pH 3, 5, 7, and 9] were mixed with vanillin (300 mg/L) in Eppendorf tubes (1 mL final volume) and incubated in a bath at three different temperatures, room temperature (25 °C), 37 °C, and 70 °C, for several days in duplicate. The concentration of tetrahydro-β-carboline **4a**,**b** produced from a Pictet−Spengler condensation was determined during time by RP-HPLC-fluorescence detection.

Total Antioxidant Capacity of Phenolic Tetrahydro-β-carbolines. To measure the radical scavenger activity, we have used an improved 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay introduced by Re et al. (*26*) that has already proved to work well to measure antioxidant activity (*27*). ABTS was dissolved in water to 7 mM concentration, and the ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM final concentration), allowing the mixture to stand in the dark at room temperature for 12−16 h before use. $ABTS^{\bullet+}$ radical cation was diluted with buffer PBS (5 mM, pH 7.2) to give an absorbance value of 0.7 at 734 nm. Phenolic tetrahydro-β-carbolines, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and ascorbic acid were dissolved in water at 1 mM and then used for radical scavenger assay in the signaled concentrations (between 1.67 and 20



R_1	R_2	R_3	R_4	Comp.	Aldehyde	Rt (min)	a/b	T (°C)
H	H	H	H	**1a,b**	Benzaldehyde	9.0, 9.29	4	25
OH	H	H	H	**2a,b**	Salicylaldehyde	8.9, 9.6	0.5	70
H	H	OCH_3	H	**3a,b**	Anisaldehyde	9.9, 9.97	0.9	70
H	OCH_3	OH	H	**4a,b**	Vanillin	8.3, 8.6	0.7	70
H	OCH_3	OH	OCH_3	**5a,b**	Syringaldehyde	8.3, 8.81	0.9	70

Figure 1. Condensation of L-tryptophan with phenolic aldehydes to give phenolic tetrahydro-β-carboline-3-carboxylic acid as two isomers (1*S*,3*S*-*cis*-**a** and 1*R*,3*S*-*trans*-**b**). Rt is the retention time in the RP-HPLC system, **a/b** is the ratio of isomers during the first days of reaction, and T is the temperature of reaction.

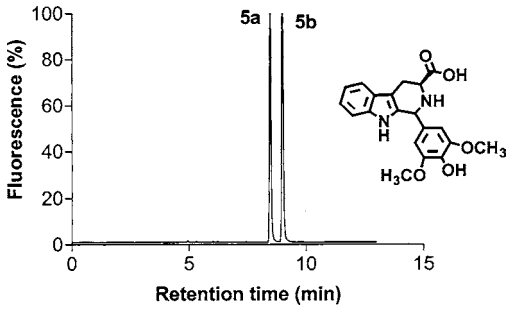


Figure 2. RP-HPLC fluorescence (270 nm excitation, 343 nm emission) chromatogram of syringaldehyde-derived tetrahydro-β-carboline (**5a**,**b**). See Materials and Methods for chromatographic conditions.

μM, final concentration) (total volume = 3 mL) by measuring the quenching of the ABTS radical (absorbance at 734 nm) as a function of time. The antioxidant capacity was measured in comparison with Trolox, the water soluble vitamine E analogue, as standard.

RESULTS AND DISCUSSION

L-Tryptophan reacted with aromatic and phenolic aldehydes such as benzaldehyde, salicylaldehyde, anisaldehyde, vanillin, and syringaldehyde in aqueous−acidic media affording the corresponding phenolic-derived tetrahydro-β-carboline-3-carboxylic acids. This reaction may occur as illustrated in **Figure 1** through a Pictet−Spengler intramolecular cyclization of the Schiff base that affords 1,3-disubstituted tetrahydro-β-carbolines containing different substituents at C-1 of the tetrahydropyrido ring depending on the aldehyde involved. 1,3-Disubstituted tetrahydro-β-carbolines arising from L-tryptophan and aromatic aldehydes gave two diastereosiomers (1*S*,3*S*-**a** and 1*R*,3*S*-**b**) that were separated by RP-HPLC (**Figure 2**). Diastereoisomer assignment was based on positive NOE of H-1 and H-3 signals in the cis isomer (**a**). H-1 signals corresponding to the cis isomer appeared at higher field than in the trans isomer, whereas C-1 and C-3 NMR signals corresponding to the trans isomer appeared at higher field that those of the cis isomer, in agreement with the expected pattern for 1,3-disubstituted tetrahydro-β-carbolines (*25*).

With the exception of benzaldehyde, this reaction required heating to significantly proceed to the cyclized products. The cis/trans diastereoisomeric ratio (**a**/**b**) for phenolic tetrahydro-β-carboline-3-carboxylic acid (syringaldehyde, salicylaldehyde, vanillin, and anisaldehyde) was lower than that of benzaldehyde, probably also influenced by the high temperature of reaction. As expected, the two isomeric compounds separated by RP-HPLC gave similar mass spectra under electrospray ionization (ESI) (**Figure 3**) with the protonated molecular ion $(M + H)^+$ and a neutral loss of 73 amu, which is characteristic of the mass spectrometry fragmentation of tetrahydro-β-carboline-3-carboxylic acids (*28*).

The above results suggest a potential formation of phenolic-derived tetrahydro-β-carboline-3-carboxylic acid under conditions of food processing or cooking as a result of the reaction between tryptophan and phenolic aldehydes. In previous studies, we have reported that short aliphatic aldehydes such as acetaldehyde and formaldehyde easily reacted with tryptophan under mild conditions (pH, temperature) providing tetrahydro-β-carbolines (*16*, *22*). Aromatic aldehydes such as those considered here may occur naturally in plant-derived and processed foods and/or can be used as flavoring agents (*23*). It is likely that those aldehydes may release tetrahydro-β-carbolines following a Pictet−Spengler reaction with available free tryptophan. In this regard, **Figure 4** illustrates the effect of temperature and pH on the formation of the vanillin−tetrahydro-β-carboline derivative **4a**,**b**. This reaction was highly accelerated in low pH and at high temperature. This behavior is quite similar to that of other tetrahydro-β-carbolines; however, in this case it needed much stronger conditions to significantly proceed than when shorter aliphatic aldehydes such as formaldehyde or acetaldehyde were involved. As a result, a more difficult formation of phenolic tetrahydro-β-carbolines than aliphatic tetrahydro-β-carbolines is expected in foods or food processing under mild conditions. Further research might address the possible occurrence of these new compounds in foods.

Currently, it is well recognized that phenolic compounds in foods possess several interesting biological and chemical properties such as antioxidant activity and the ability to scavenge reactive oxygen species (*29*−*32*). As a consequence, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, and inflammation. Because the above β-carbolines contain phenolic substituents that might be of further significance, we have assayed the total antioxidant capacity of these novel phenolic-derived tetrahydro-β-carboline-3-carboxylic acids in their ability to scavenge free radicals (**Figure 5**). The activity of tetrahydro-β-carbolines as radical scavengers and antioxidants was measured in the $ABTS^{\bullet+}$ radical cation assay (*26*) by measuring the reduction of the radical cation $ABTS^{\bullet+}$ produced by an antioxidant. Phenolic tetrahydro-β-carbolines **1**−**5** exhibited greater reactivity as antioxidants than the classical antioxidant ascorbic acid and the water soluble vitamin E analogue, Trolox. The antioxidant capacity of the alkaloids measured as Trolox equivalent antioxidant capacity (TEAC, mM) at a fixed time point of 5 min (TEAC is defined as the concentration of Trolox required to scavenge the radical $ABTS^{\bullet+}$ to an extent equivalent to that of a 1 mM concentration of the antioxidant) gave values of 2.1 mM (**1**), 2.64 mM (**2**), 1.88 mM (**3**), 2.04 mM (**4**), and 2.46 mM (**5**), which were much higher than those for ascorbic acid (1.01 mM) and Trolox (1 mM). The TEAC value measured for phenolic tetrahydro-β-carbolines is higher than the TEAC value for other tetrahydro-β-carbolines showing antioxidant activity and lacking phenolic substituents (*33*). The presence of hydroxyl groups attached to the aromatic ring increased this activity. Therefore, these alkaloids are good antioxidants and radical scavengers, at least as seen here in this assay. Further studies will be needed to confirm this finding under other conditions and assays.

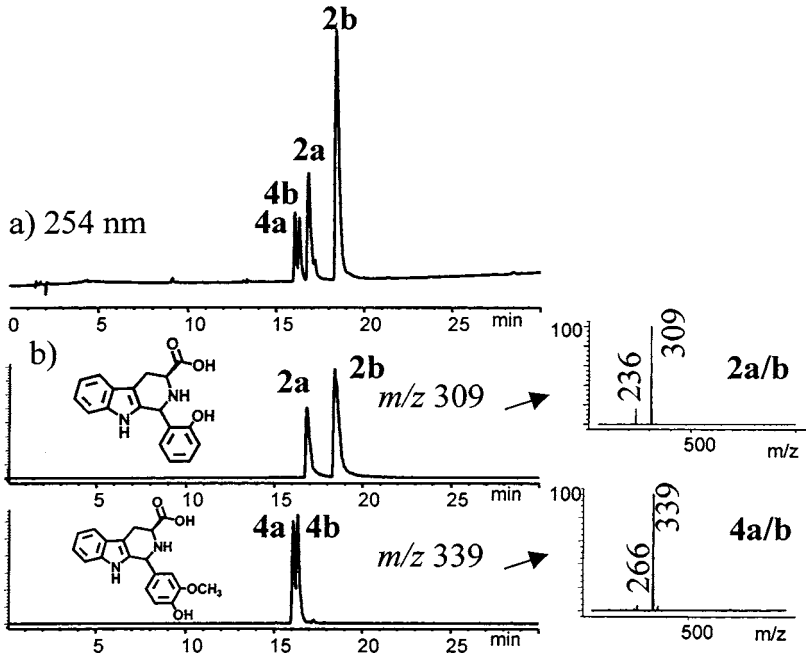


Figure 3. RP-HPLC electrospray ionization (ESI-reconstructed ion chromatogram) and mass spectra of a solution made of tetrahydro-β-carboline-3-carboxylic acid derived from salicylaldehyde (**2**) and vanillin (**4**).

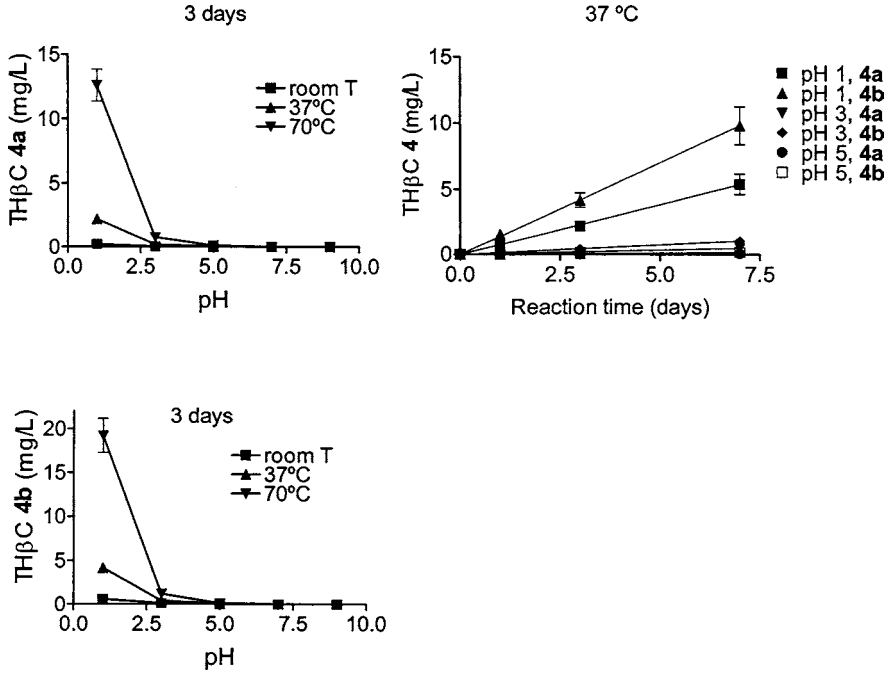


Figure 4. Formation of vanillin-derived tetrahydro-β-carboline-3-carboxylic acid as a function of pH and temperature. Values are the average of duplicate measurements, and bars indicate the standard deviation.

These and our previous results evidence the formation of tetrahydro-β-carboline-3-carboxylic acids through Pictet−Spengler chemical condensation between tryptophan and aldehydes in conditions that occur during food production, processing, and storage. Thus, dietary tetrahydro-β-carbolines conform a likely exogenous source of these alkaloids that may affect their reported endogenous presence in biological systems (*3*−*8*, *34*). Although several studies have dealt with the possible biological activity of tetrahydro-β-carbolines and β-carbolines, there is still a need for its full delineation. In this paper, we have shown that this class of alkaloids might act as good antioxidants and free radical scavengers.

CONCLUSIONS

L-Tryptophan reacted with aromatic and phenolics aldehydes such as benzaldehyde, vanillin, salicylaldehyde, anisaldehyde, and syringaldehyde in acidic−aqueous conditions affording phenolic tetrahydro-β-carboline-3-carboxylic acid. As a result, those phenolic tetrahydro-β-carbolines are likely to occur in foods or food processing and cooking if allowed appropriate conditions such as low pH, relatively high temperature, and presence of the needed precursors. The new phenolic tetrahydro-β-carbolines showed good antioxidant properties that were much higher than those determined for ascorbic acid and the water soluble vitamin E analogue, Trolox.

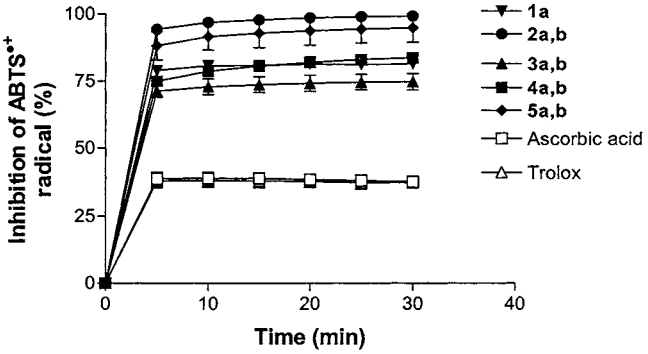


Figure 5. Radical scavenger activity (total antioxidant activity) of phenyl and phenolic tetrahydro-β-carbolines **1**–**5** as well as ascorbic acid and Trolox in the ABTS assay measured as a percentage of the inhibition of the radical ABTS$^{\bullet+}$ (absorbance at 734 nm). Date are the average of quadruplicates in 10 μM concentration. Compounds **2**–**5** were mixed isomers (*cis*, *trans*), and **1** was the *cis* isomer.

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